

学位論文要旨

Research on allelopathy of Vietnamese tea (*Camellia sinensis* (L.) Kuntze) and demonstration of caffeine as putative allelochemical in action

ベトナム茶のアレロパシーに関する研究とアレロケミカルとしてのカフェインの推定

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Herbicides have become indispensable for crop protection from damage of various weeds, weed management has been mainly depending on synthetic herbicides. Widespread use of synthetic herbicides has resulted in herbicide-resistant weeds and the unsafe of it. Reducing the contamination of those herbicide to the environment is important step toward sustainable agricultural. In recent years, allelopathic species and allelochemicals have been applied as an alternative weed management method. Addition, the increasing possibilities for developing bio-herbicides from plants that could be used for weed control to minimize the heavy reliance on synthetic herbicides. Tea (*Camellia sinensis* (L.) Kuntze) is one of the most abundant plants in Vietnam by many functions in food and beverage. Many previous reports showed that caffeine and catechins can be allelochemical candidates in tea. However, the contribution of each compound to the total inhibitory activity of tea and their action pathways has not been clarified so far. Our study was conducted to evaluate the allelopathic potential of several tea samples from Vietnam.

The objectives of this study were: (i) to screen of allelopathic activity of tea leaves, (ii) to identify caffeine and evaluate the contribution of caffeine in the allelopathic activity of tea leaves, (iii) to evaluate the plant growth inhibitory activity of caffeine on the growth of crop and weed species, (iv) to evaluate the phytotoxic activity and concentration of caffeine from tea residues in three kinds of the soils and tea garden soil, (v) to screening of allelopathic potential of callus of Yabukita, Tsubaki and Sazanka tea and identify caffeine from callus of them.

All tea samples have shown a dose-dependent inhibitory effect on the radicle and hypocotyl growth of lettuce seedling. Among the seven tea samples, the leachates from Vina tea-green tea showed the highest inhibition on the radicle growth of lettuce seedlings with 50% suppression at 0.12 mg dry leaves/ml of agar, it is suggested

that the highest inhibitory activity on the growth of lettuce seeds was observed in Vinatea- green tea sample. In contrast, black tea had the lowest plant growth inhibitory activity ($EC_{50} > 10$ mg/ml of agar) compared to green, oolong and dried tea leaves (unprocessed tea samples).

The highest content of caffeine was found in the green tea samples (20.7-38.2 mg/ml) with the maximum content in Vinatea-green tea. The lowest caffeine content (20.7 mg/ml) was found in dried tea leaves. The difference in caffeine concentrations of tea samples may be related to the time of tea harvesting, variety, and the manner of tea processing. Caffeine had the specific activity EC_{50} of 75 and 183 μ g/ml for lettuce radicle and hypocotyl growth respectively. Based on the value of the total activity, the contribution of caffeine can explain inhibitory activity by crude tea extracts in group of green and oolong tea sample. And for dried leave, Vinatea- oolong tea and Vinatea-black tea, we assume the possible effect of also other compounds such as catechins and polyphenols.

Moreover, the allelopathic activity of pure caffeine and aqueous tea extracts was highly selective on the growth of different plant species. Caffeine has been shown to have a strong allelopathic potential on seed germination and growth of some weeds and crops.

The recovery concentration of caffeine extracted with methanol from soil treated pure caffeine and green tea residue with EC_{50} value were about 254 ppm and 169 ppm in Fluvisols soil, and 746 and 663 ppm in Andosols soil, respectively. These results indicated that caffeine was absorbed by Fluvisols soil in a significant amount compared to Andosols and Sands soils. This observation perhaps due to the physical properties of soil and particularly its texture. Among three soils, caffeine concentration from tea residue was the highest in Andosols. Therefore, soil type significantly affects allelopathic activity.

The caffeine concentration in soil has a positive correlation with radicle growth inhibition of lettuce seeds at ($r=0.716^{***}$; $p < 0.01$). The concentration of caffeine found from tea farm soil was 0.14 μ g/g soil. Detection of caffeine in soil indicated that caffeine could directly affect the growth of plants in soil and the effect depends on concentration.

In addition, the study of allelopathic activity at the cellular level using the main Japanese tea cultivars. Callus of Yabukita tea showed strong inhibitory effect with the rate of 85% at 10 mg of both dried leaves and callus per 10 ml of agar on the growth of lettuce seedling. It was found that caffeine concentration in Yabukita callus was 19.2 μ g/g.

The present study revealed that caffeine is responsible for the allelopathic effect of tea extracts and the selective weed inhibitory properties of aqueous extraction of Vinatea-green tea and caffeine. Addition, soil type significantly affects caffeine allelochemical activity. Although, caffeine concentration was found in a small amount but our study has proved the presence of caffeine in the soil of the tea garden and in calluses suggesting it could be agriculturally significant for sustainable weed management.